

**AWARD NUMBER:** W81XWH-16-1-0261

**TITLE:** Targeting Tumor-Initiating Cells for the Therapeutics of Breast Cancer

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**REPORT DATE:** October 2017

**TYPE OF REPORT:** Annual

**PREPARED FOR:** U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE October 2017		2. REPORT TYPE Annual		3. DATES COVERED 15 Sep 2016 - 14 Sep 2017	
4. TITLE AND SUBTITLE  Targeting Tumor-Initiating Cells for the Therapeutics of Breast Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-16-1-0261	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)  Songhai Chen (PI)  E-Mail: Songhai-chen@uiowa.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  The University of Iowa, Iowa City, IA 52242				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT  HER2 <sup>+</sup> breast cancers are a highly aggressive form that occurs in 20-30% of metastatic breast cancers and correlates with poor prognosis. HER2 <sup>+</sup> breast cancers are usually treated with HER2-targeted therapy, but cancers quickly develop resistance to the therapy within 1 to 2 years. The mechanisms underlying resistance remain largely unknown but are attributed to a reservoir of stem-cell-like, tumor-initiating cells (TICs). Under the pressure of current therapies, these cells have a survival advantage and may escape therapies, and so likely account for drug resistance, tumor recurrence, and metastasis. This proposal aims to determine the role of Gi/o-coupled receptors (Gi/o-GPCR) signaling in regulating the tumorigenicity of TICs to drive HER2 <sup>+</sup> breast cancer growth and metastasis, and confer drug resistance to the HER2-targeted therapy. Our studies thus far have demonstrated that Gi/o-GPCR signaling is essential for the initiation and progression of HER2-induced mammary tumors in mice, and HER2-mediated human breast cancer cell growth and migration in vitro. Moreover, we provided the evidence that Gi/o-GPCRs drive tumor progression at least in part through enhancing the tumorigenicity of TICs. The proposal was terminated early because of partial overlapping with a NCI-funded R01 proposal. Nevertheless, findings from these studies have laid the foundation for further investigation of the function and mechanisms of Gi/o-GPCRs in driving HER2 breast cancer.					
15. SUBJECT TERMS HER2+ breast cancer, G protein coupled receptors, signal transduction, ,HER2-targeted therapy, drug resistance					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT  Unclassified	18. NUMBER OF PAGES  11	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

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- 1. INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

HER2<sup>+</sup> breast cancers are a highly aggressive form that occurs in 20-30% of metastatic breast cancers and correlates with poor prognosis. HER2<sup>+</sup> breast cancers are usually treated with HER2-targeted therapy, but cancers quickly develop resistance to the therapy within 1 to 2 years. The mechanisms underlying resistance remain largely unknown but are attributed to a reservoir of stem-cell-like, tumor-initiating cells (TICs). This proposal aims to determine the role of Gi/o-coupled receptors (Gi/o-GPCR) signaling in regulating the tumorigenicity of TICs to drive HER2<sup>+</sup> breast cancer growth and metastasis, and confer drug resistance to the HER2-targeted therapy. Specifically, we will determine 1) how Gi/o-GPCR signaling enhances tumorigenicity of TICs in human HER2<sup>+</sup> breast cancer; 2) if upregulated Gi/o-GPCR signaling drives breast cancer progression through a c-Src-EGFR/HER2 signaling axis; and 3) if inhibiting Gi/o-GPCR signaling enhances sensitivity to HER2-targeted therapy, in both trastuzumab-sensitive and -resistant HER2<sup>+</sup> cancer cells.

- 2. KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

HER2<sup>+</sup> breast cancer, G protein coupled receptors, signal transduction, HER2-targeted therapy, drug resistance

- 3. ACCOMPLISHMENTS:**

**What were the major goals of the project?**

The major goals of the project include the following:

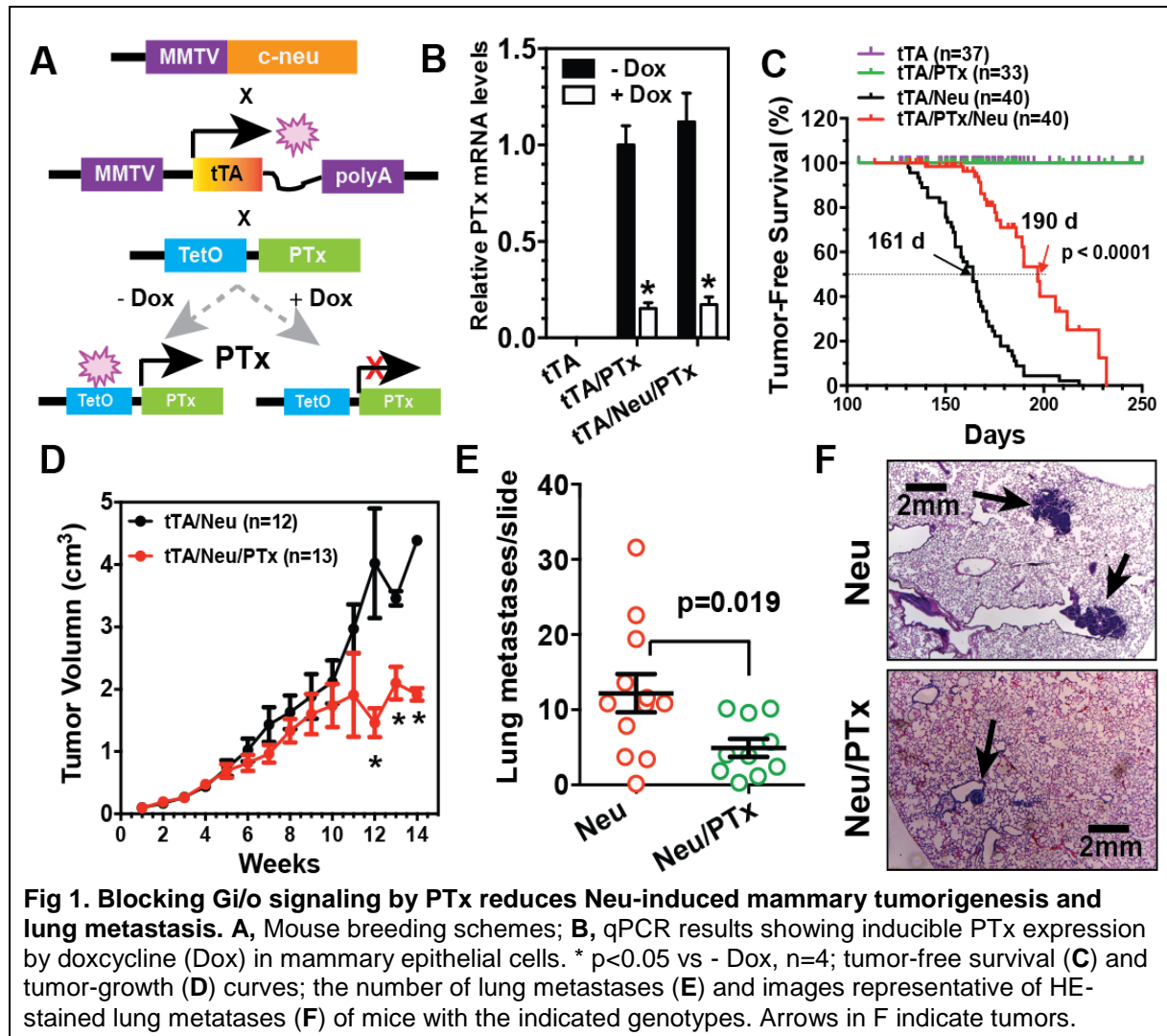
1. Establish the roles of Gi/o-GPCR signaling in HER2-driven TIC tumorigenicity and tumor progression (Months 1-24).
2. Uncover the molecular mechanisms by which upregulated Gi/o-GPCR signaling stimulates tumorigenicity of TICs, driving cancer progression (Months 13-30).
3. Evaluate the potential of targeting Gi/o-GPCR signaling as a new way to ablate TICs, enhancing the efficacy of HER2-targeted therapy in naïve or trastuzumab-resistant HER2<sup>+</sup> cancer cells (Months 25-36).

**What was accomplished under these goals?**

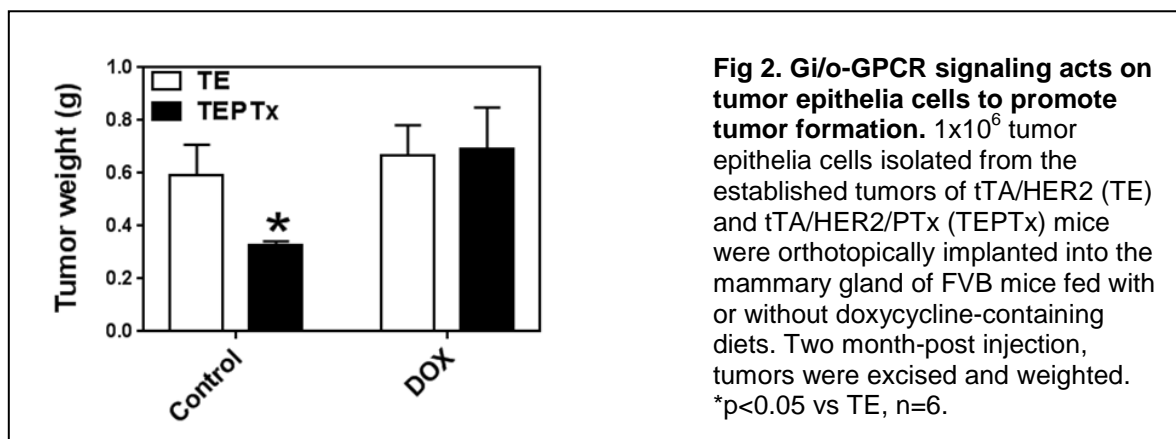
The project was terminated early due to partial overlapping with a NCI-funded R01. Nevertheless, we have accomplished the following tasks during the period of 09/15/2016 to 04/30/2017.

1. Establish the role Gi/o-GPCR signaling in mediating HER2-driven tumor progression in mice.  
Our results showed that blocking Gi/o-GPCR signaling by expressing pertussis toxin (PTx) in mammary gland delayed tumor onset and slowed primary tumor growth induced by HER2/neu overexpression (Fig. 1A-D). Moreover, HER2/neu-driven tumor metastasis was significantly reduced by PTx (Fig. 1E-F). Further studies of transplanting tumor cells into FVB

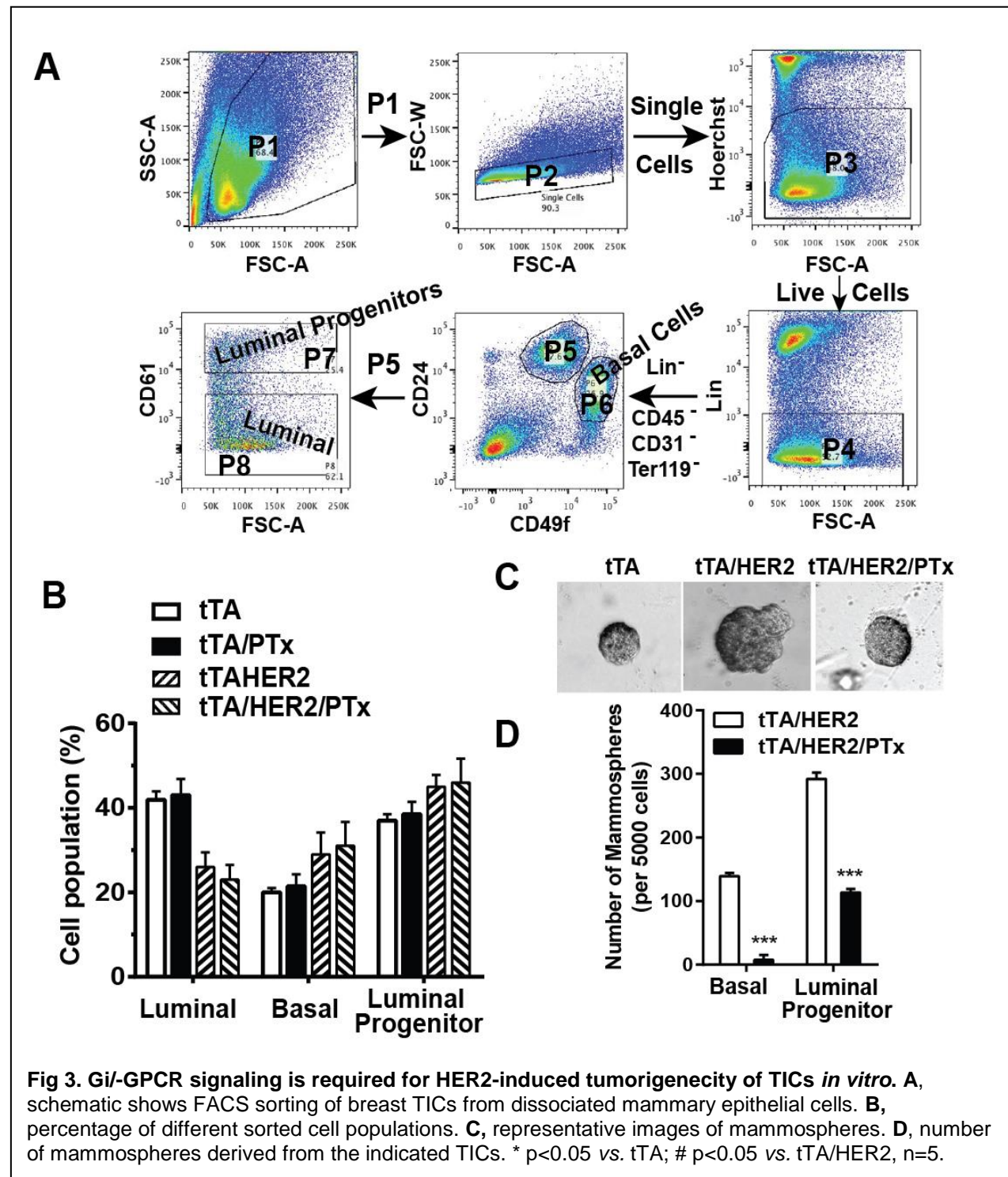
mice showed that tumors formed from tumor cells expressing PTx and HER2/neu were significantly smaller than those from tumor cells expressing HER2/neu alone, and this difference in tumor formation was abolished by blocking PTx expression via treating mice with doxycycline (Fig. 2). These findings demonstrated that the inhibitory effect of PTx on tumor progression was likely due to direct inhibition of Gi/o-GPCR signaling in tumor epithelial cells.



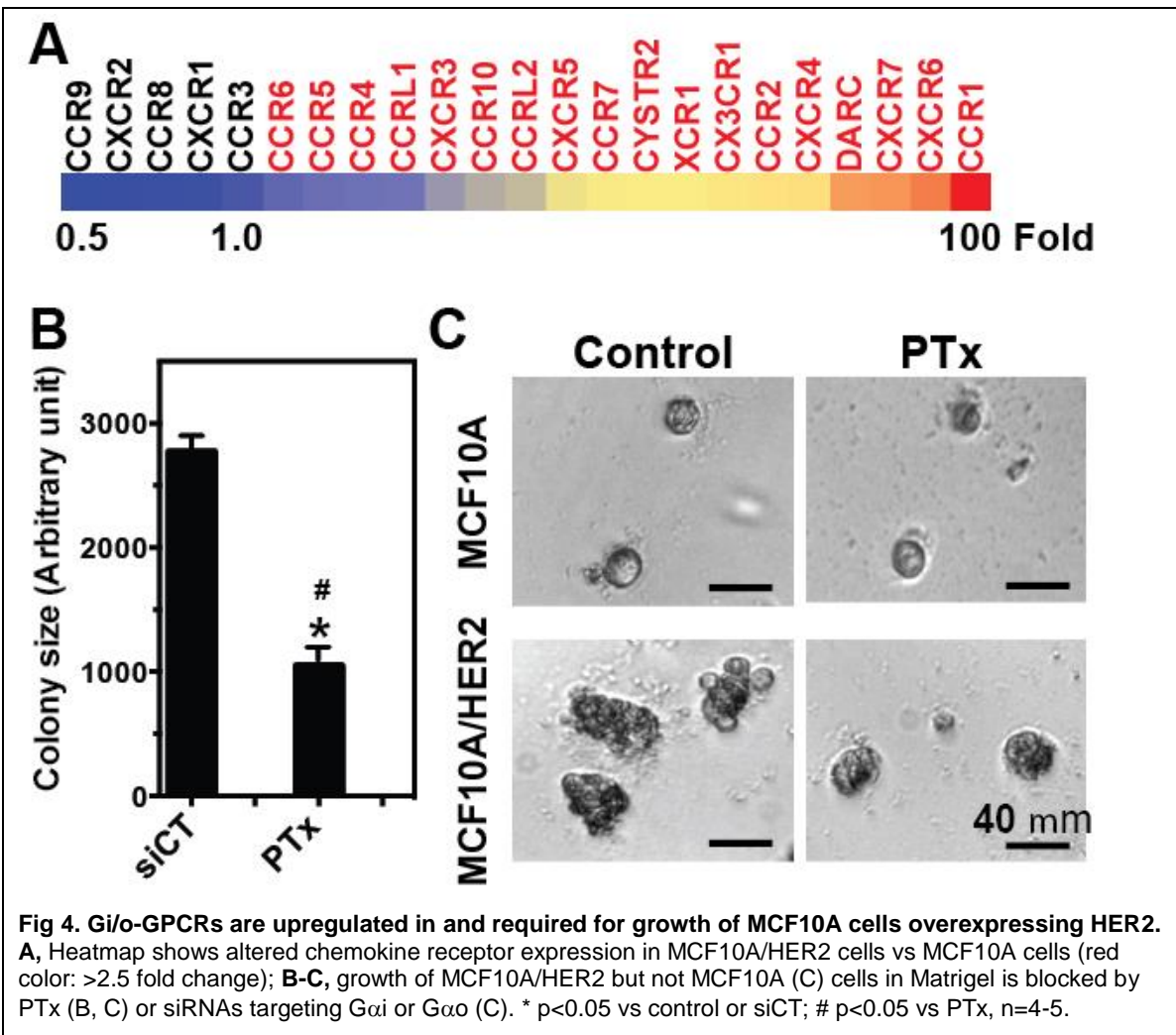
**Fig 1. Blocking Gi/o signaling by PTx reduces Neu-induced mammary tumorigenesis and lung metastasis.** **A**, Mouse breeding schemes; **B**, qPCR results showing inducible PTx expression by doxycycline (Dox) in mammary epithelial cells. \* p < 0.05 vs -Dox, n=4; tumor-free survival (**C**) and tumor-growth (**D**) curves; the number of lung metastases (**E**) and images representative of HE-stained lung metastases (**F**) of mice with the indicated genotypes. Arrows in **F** indicate tumors.



2. Demonstrate that Gi/o-GPCR signal is required for the tumorigenicity of TICs *in vitro*. Using the well-known cell surface makers for basal cells and luminal progenitor cells, we identified the TIC population in mammary epithelial cells from estrous-cycle matching, 4-month old mice expressing HER2/neu or HER2/neu plus PTx mice (Fig. 3A). Co-expression of PTx with Neu did not affect the number of basal or luminal progenitors (Fig. 3B), but significantly impaired Neu-mediated tumorsphere formation, particularly from basal cells (Fig. 3C-D). These data suggest that Gi/o-GPCR signaling is hyperactivated in TICs, promoting HER2/neu-mediated tumorigenic TIC growth.



3. Demonstrate that Gi/o-GPCRs are upregulated by HER2 overexpression and are required for human breast cancer cell growth. Using qPCR-based GPCR arrays to profile GPCR expression, we found that many chemokine receptors were upregulated in HER2-overexpressing MCF10A cells, as compared to the parental MCF10A cells (Fig.4A). Moreover, treatment with PTx blocked the growth of MCF10A overexpressing HER2 but not the parental MCF10A cells (Fig. 4B-C). These data indicate that Gi/o-GPCR signaling also supports growth of human HER2<sup>+</sup> breast cancer cells.



**What opportunities for training and professional development has the project provided?**

Nothing to Report.

**How were the results disseminated to communities of interest?**

Nothing to Report.

**What do you plan to do during the next reporting period to accomplish the goals?**

Nothing to Report.

- 4. IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

**What was the impact on the development of the principal discipline(s) of the project?**

Our studies have demonstrated that Gi/o-GPCR signaling plays a critical role in promoting HER2-driven breast cancer progression. These findings have laid the foundation for further investigation of the underlying mechanisms by which Gi/o-GPCRs regulate HER2 signaling to promote breast cancer progression, and testing the potential of targeting Gi/o-GPCRs to enhance the efficacy of HER2-targeted therapies.

**What was the impact on other disciplines?**

Nothing to Report.

**What was the impact on technology transfer?**

Nothing to Report.

**What was the impact on society beyond science and technology?**

Nothing to Report.

**5. CHANGES/PROBLEMS:**

The project was terminated early due to partial overlapping with a NCI-funded R01.

- 6. PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

- **Publications, conference papers, and presentations**

Nothing to report.



- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### What individuals have worked on the project?

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change.”*

*Name:* Songhai Chen

*Project Role:* PI

*Researcher Identifier (e.g. ORCID ID):* [0000-0001-7316-7125](https://orcid.org/0000-0001-7316-7125)

*Nearest person month worked:* 8

*Contribution to Project:* Dr. Chen has provided overall administration and direction of the project.

*Name:* Prasanna Vaddi

*Project Role:* Postdoc

*Researcher Identifier (e.g. ORCID ID):*

*Nearest person month worked:* 8

*Contribution to Project:* Dr. Vaddi has been involved in characterizing the tumor progression of the transgenic mice and TICs, and contributed to the generation of the data in Figs. 1 and 3.

*Name:* Khemraj Baghel

*Project Role:* *Postdoc*  
*Researcher Identifier (e.g. ORCID ID):*  
*Nearest person month worked:* 5

*Contribution to Project:* *Dr. Baghel has been involved in generating the orthotopically transplanted mouse models of tumor cells and characterizing TICs, and contributed to the generation of the data in Figs. 2 and 3*

*Name:* *Jianling Bi*  
*Project Role:* *Postdoc*  
*Researcher Identifier (e.g. ORCID ID):*  
*Nearest person month worked:* 2

*Contribution to Project:* *Dr. Bi has been involved in characterizing GPCRs in human breast cancer cells, and contributed to the generation of the data in Fig. 4.*

*Name:* *Maddi Lensing*  
*Project Role:* *Research Intern*  
*Researcher Identifier (e.g. ORCID ID):*  
*Nearest person month worked:* 3

*Contribution to Project:* *Ms. Lensing has contributed to the breeding and characterization of the transgenic mice*

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

An R01 application was funded (05/01/2017-04/30/2022) from NCI to the PI, Dr. Songhai Chen after the current funding was terminated on April 30<sup>th</sup> 2017. This R01 partially overlaps with the DOD grant.

**What other organizations were involved as partners?**

Nothing to report.

**8. SPECIAL REPORTING REQUIREMENTS**

**Not applicable.**

- 9. APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

None.